

# Assessing and Mitigating Simulated Population-Level Effects of 3 Herbicides to a Threatened Plant: Application of a Species-Specific Population Model of *Boltonia decurrens*

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**Abstract:** Extrapolating from organism-level endpoints, as generated from standard pesticide toxicity tests, to populations is an important step in threatened and endangered species risk assessments. We apply a population model for a threatened herbaceous plant species, *Boltonia decurrens*, to estimate the potential population-level impacts of 3 herbicides. We combine conservative exposure scenarios with dose–response relationships for growth and survival of standard test species and apply those in the species-specific model. Exposure profiles applied in the *B. decurrens* model were estimated using exposure modeling approaches. Spray buffer zones were simulated by using corresponding exposure profiles, and their effectiveness at mitigating simulated effects on the plant populations was assessed with the model. From simulated exposure effects scenarios that affect plant populations, the present results suggest that *B. decurrens* populations may be more sensitive to exposures from herbicide spray drift affecting vegetative stages than from runoff affecting early seedling survival and growth. Spray application buffer zones were shown to be effective at reducing effects on simulated populations. Our case study demonstrates how species-specific population models can be applied in pesticide risk assessment to bring organism-level endpoints, exposure assumptions, and species characteristics together in an ecologically relevant context. *Environ Toxicol Chem* 2018;37:1545–1555. © 2018 SETAC

**Keywords:** Population modeling; Individual-based model; Endangered Species Act; Pesticide risk assessment; Runoff; Drift

## INTRODUCTION

Agricultural landscapes are interwoven with natural and seminatural habitats where plant species listed as threatened or endangered under the US Endangered Species Act may be found. Species in such habitats have the potential to be exposed to pesticides entering the habitat via spray drift or runoff from adjacent treated fields (de Snoo and van der Poll 1999; Egan et al. 2014a). However, exposures may be limited to areas directly adjacent to treated fields (Marrs and Frost 1997; Brain et al. 2017), and levels of effects from such exposures may vary depending on the species of interest, the pesticide applied, the species community, and other environmental factors that may interact with potential pesticide effects (Marrs et al. 1991; Marrs and Frost 1997; Boutin et al. 2012, 2014). Given these complex

interactions, accurately assessing risks of pesticides to species listed under the US Endangered Species Act is a challenging endeavor.

The pesticide data available for listed species risk assessments usually include toxicity endpoints as tested with a suite of standard species, spatial and temporal use patterns of the pesticide and estimates of potential exposure levels, and the geographical range over which the species occurs. Both geographical use patterns and species occurrences are often only available at a coarse level, for example, by US county. If registered applications of a pesticide and a species' range do not overlap, it can be concluded that the pesticide is unlikely to affect the species (National Research Council 2013; Brain et al. 2015). If a potential overlap does occur, the available endpoints and species characteristics need to be considered to ensure that the species is not adversely affected by use of the pesticide. Exposure and effects levels may differ between compounds and species, but the interaction with a species' life history and environment result in similarities in risk assessments for different pesticides. The decurrent false aster (*Boltonia decurrens*) is an

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example of an herbaceous plant species listed as threatened under the US Endangered Species Act (US Fish and Wildlife Service 2012). The species occurs in the historic floodplain of the Illinois River and just south of the confluence of the Illinois and Missouri Rivers. Large parts of the historic floodplain have been converted to agriculture (Sparks et al. 1998; Smith et al. 2005), resulting in potential overlap of the species' range and agricultural areas as assessed at the county level.

To assess potential risks of pesticide exposures to the species' populations that may exist near an agricultural application, the combination of pesticide toxicity, exposure levels, and the species' characteristics has to be considered. Population modeling can be used as a tool to estimate potential risk from pesticides to sensitive populations by integrating multiple sublethal and lethal effects simultaneously and by accounting for differences in species' life histories (Schmolke et al. 2010a; Galic et al. 2010; Forbes et al. 2016). In the present study, we apply a species-specific population model of *B. decurrens* to conduct experiments that can inform herbicide risk assessments (Schmolke et al. 2017).

In the present study, we combine exposures and effects from simulated spray drift and runoff to simulated populations of *B. decurrens*. We compare the population-level effects of 3 herbicides, atrazine (photosystem II inhibitor), mesotrione (4-hydroxyphenylpyruvate dioxygenase inhibitor), and S-metolachlor (long-chain fatty acid synthesis inhibitor), on populations of the plant and assess exposure scenarios assuming exposure from spray drift and runoff separately and in combination. We then consider how effects as observed in the simulated populations could be mitigated by modeling spray setback distances (e.g., buffers) where herbicide application is removed by a given distance from the simulated *B. decurrens* habitat.

## METHODS

### Species

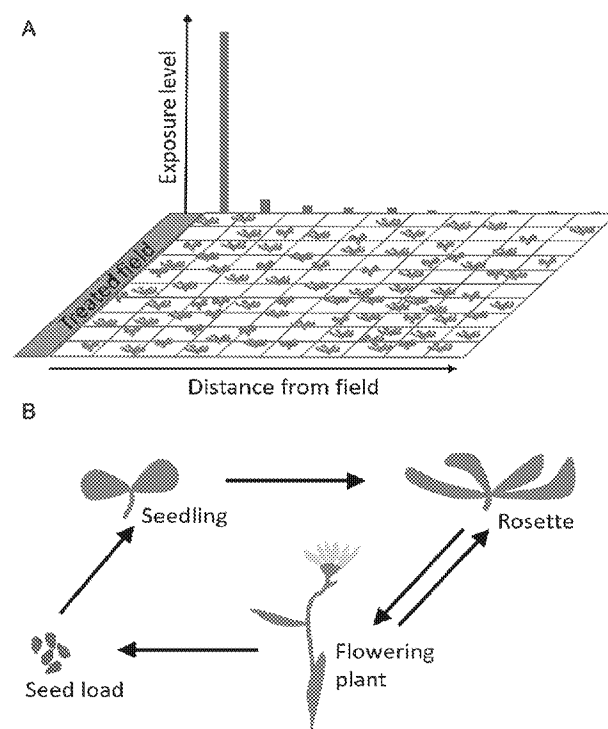
The decurrent false aster (*B. decurrens*, Asteraceae) is an herbaceous plant species listed as threatened under the US Endangered Species Act for its whole range (Harrison 1988). The species historically occurred throughout the floodplain of the Illinois River and the Missouri River (USA) just south of the confluence of the 2 rivers. Large parts of the floodplain have been drained for agriculture and development along the rivers, and the construction of locks and dams has altered the temporal pattern of floods in the remaining flood areas (Sparks et al. 1998; Smith et al. 2005). *Boltonia decurrens* is a fugitive species that is flood-resistant and can populate flood areas in high densities (Smith and Moss 1998; Smith et al. 1998). In the absence of floods, it is quickly outcompeted by other species. The species is a short-lived perennial with 2 distinct life-history pathways. Mature plants reproduce vegetatively by growing rosettes from their roots. The rosettes grow independently after the parental plant dies after seed set in the fall and bolt and flower in the following year. Seeds germinate throughout the year, with peak germination in April and May (Moss 1997; Baskin and Baskin

2002). Seedlings emerging in the spring generally flower, produce seeds, and die in the same year. Fall seedlings remain in rosette stage over winter and mature in the following year (Smith and Keevin 1998).

### Model summary

A detailed description of the individual-based population model for *B. decurrens* was provided in a previous publication (Schmolke et al. 2017), including a TRACE (transparent and comprehensive ecological model) documentation (Schmolke et al. 2010b; Grimm et al. 2014). In the present study, we provide a summary of the model. Changes applied to the model to allow for exposure via runoff and drift affecting seedling emergence and vegetative vigor endpoints, respectively, and multiple exposures and pathways are described in detail in the Supplemental Data (section 1). The model code and input files are also provided in the Supplemental Data.

The population model is implemented as an individual-based model using the modeling software NetLogo, Ver 5.3.1 (Wilensky 1999). A graphical overview of the model is given in Figure 1. Each plant is represented individually by its location (x- and y-coordinates), life stage (seedling, rosette, or flowering plant), and biomass. The habitat ("world" in NetLogo jargon) is represented as an area of  $20 \times 20 \text{ m}^2$  and subdivided into grid cells with 2-m edge length. A time step in the model represents a



**FIGURE 1:** Overview of the *Boltonia decurrens* population model. (A) Plants are represented individually with a location in the habitat ( $20 \times 20 \text{ m}^2$ ). Herbicide exposures are simulated once a year, with exposure levels varying dependent on the distance (in 2-m bins) to the treated field. (B) Plants are modeled as 3 distinct life stages: seedlings, rosettes, and flowering plants. Seeds are represented not individually but only as their presence or absence in a given grid cell in the model.

week, and a year is represented as 30 time steps, reflecting the vegetative period between early April and late October. Dormancy is assumed in winter during which no growth occurs, but overwintering rosettes may experience background mortality. For the simulations shown in the present study, 15 yr of simulations were analyzed after allowing for a 5-yr establishment period (see *Data collection and analysis from simulations*).

Seeds are represented not individually in the model but as “seed load”; that is, each habitat grid cell with a seed load is assumed to contain enough seeds to establish a stand of *B. decurrens* plants. This assumption is based on the prolific seed production of flowering plants (50 000 or more seeds produced per plant [Smith and Keevin 1998]). If a cell does not have a seed load, no seedlings emerge in the cell. Simulated *B. decurrens* plants emerge as seedlings over an 8-wk period in the model (corresponding to April and May). The plants remain in the seedling stage for 2 wk, then transition to the rosette stage. Seedling and rosette growth is simulated according to the Gompertz function applied to the relative growth rate of the plants. This growth model results in an initial exponential phase of growth, transitioning to linear growth, and finally an asymptotic growth phase. Growth ceases once the plants start flowering. From the plant weight, plant radius and (in the case of flowering plants) plant height are calculated. Plants reach the flowering stage between weeks 19 (mid-August) and 28 (early October) as soon as their biomass exceeds the flowering threshold. Plants smaller than the threshold in week 28 are considered recumbent rosettes and remain in the rosette stage until the following year. Flowering plants reproduce by the production of vegetative rosettes located around the circumference of the parental plant and via seed production. The number of vegetative rosettes produced is dependent on plant size; that is, larger plants produce more vegetative rosettes. Vegetative rosettes overwinter and reach the flowering stage in the following year. The high seed production of *B. decurrens* plants is represented by assuming that a habitat grid cell is supplied with enough seeds by a single flowering plant to result in a seed load in the cell. Flowering plants can supply seed loads to neighboring cells with a probability declining with distance. All flowering plants die at the end of the simulated year. For the purposes of the model, we assume that *B. decurrens* does not form a seed bank, and all seeds are simulated to germinate in their first year. The seeds can be viable for several years under laboratory conditions (Baskin and Baskin 2002), but seeds recovered from soil cores in the field were observed to have very low germination rates (Smith et al. 2005). In the context of our model, a seed bank would only be relevant in case no flowering and seed production would occur for more than one season because the seed production by a single flowering plant is assumed to be sufficient to saturate the seed load within a cell in the model.

Seedlings and rosettes are assumed to compete for light with their nearest neighbor in rosette or flowering stage. In the model, the nearest neighbor is determined by the distance between the coordinates of the plants within the same habitat grid cell. Larger plants are assumed to shade smaller plants, inhibiting their growth, but not vice versa. Growth inhibition as a

result of shading is proportional to the overlap between the 2 plants as calculated from their coordinates and radii. Seedling establishment is suppressed in areas already occupied by rosettes. Seedlings are assumed not to be impacted in their growth by other seedlings because a maximum seedling density is assumed in the model and can occur in habitat grid cells void of other vegetation. Because flowering plants do not grow anymore, they are not impacted by competition or other factors that impact plant growth. However, flowering plants can shade and, thus, impact growth of rosettes.

Competition with co-occurring vegetation other than *B. decurrens* plants is represented in the model as a single factor, the vegetation index. A higher vegetation index represents more shading of *B. decurrens* plants from competing vegetation and results in reduced growth. The vegetation index is assumed to remain unchanged during the growing season if no disturbances occur. This assumption reflects similar growth rates in *B. decurrens* and its competitors. The vegetation index is a very simplified representation of other vegetation and represents the relative impact of competition by other herbaceous vegetation on *B. decurrens* plants but not vice versa. The co-occurring vegetation is assumed to be more sensitive to flooding than *B. decurrens* (Stoecker et al. 1995; Smith and Moss 1998). Accordingly, the vegetation index is reduced because of simulated flooding events. Flooding can occur during the winter dormancy period and during the simulated year. The duration of flooding determines the reduction of the vegetation index; that is, for every week of simulated flooding, the vegetation index is reduced. If flooding occurs during the simulated year, *B. decurrens* plants experience reduced growth but no direct mortality. No effects on *B. decurrens* plants are assumed if floods occur during winter dormancy. Only flood duration, not flood depth, is represented in the model because flood conditions in *B. decurrens* habitats are mostly observed as low-depth standing rainwater or floodwaters on river banks. Catastrophic floods are not considered in the simulations. Flood scenarios applied in the present study are addressed in more detail (see *Data collection and analysis from simulations*).

Herbicide exposures are simulated to affect the simulated plants in their survival probability and growth according to dose-response functions. Effects data are available from standard toxicity tests on plants. In standard regulatory seedling emergence studies, herbicides are applied to the soil at the time of seed planting. In the model, the exposure-effects relationships from the seedling emergence studies are used to simulate the effects of exposure from runoff. Data from seedling emergence studies (Organisation for Economic Co-operation and Development 2006a; US Environmental Protection Agency 2012a) include effects on seedling emergence (or germination rate), seedling survival, and growth. Effects on seedlings in the seedling emergence studies were assessed 2 wk after exposure to atrazine and 3 wk after exposure to mesotrione and S-metolachlor. In the model, effects were simulated for the duration of the corresponding seedling emergence studies (2- and 3-wk time steps, respectively) to achieve the best match with the available data. Seedlings are simulated to establish over 8 wk in the spring. Seedlings establishing during the simulated

exposure period experience effects according to dose–response functions for seedling emergence endpoints. Seedlings emerging before or after the simulated runoff exposure period were assumed not to be affected in seedling emergence endpoints. In each week, while effects are simulated to last, survival and growth rates are reduced according to the dose–response functions (see *Dose responses of 3 herbicides simulated in the model*). After the effects duration, surviving plants in the model revert to the growth rate demonstrated by unexposed plants of the same age (note that growth rate may still be affected by other factors, i.e., competition or flooding). If runoff exposure exceeds the duration of seedling stage (2 wk in the model), effects on survival and growth rate are carried over into the rosette stage.

In vegetative vigor studies, herbicides are applied via overspray to established plants (Organisation for Economic Co-operation and Development 2006b; US Environmental Protection Agency 2012b). We apply the exposure–effects relationships from these studies to simulate the potential effects of varying levels of spray drift on the *B. decurrens* plants. Vegetative vigor endpoints, that is, survival and growth of established plants, are assessed 3 wk after exposure in the vegetative vigor studies for the 3 herbicides (atrazine, mesotrione, and S-metolachlor). The effects on the *B. decurrens* plants in the model are simulated to last for the same duration. Seedlings established at the time of initial spray drift exposure are affected, as are plants in the rosette stage. Seedlings emerging after the initial week of exposure but during the following 2 wk that the effects are simulated to last are not affected. Effects on seedlings exposed to spray drift carry over into their rosette stage. After the 3-wk effects period, surviving plants revert to the unexposed growth rate.

For cases in which exposures via spray drift and runoff were simulated to occur in combination, plants affected by both exposure routes were simulated to experience an additive effect. Survival and growth rates were reduced proportionally according to the runoff exposure and dose responses derived from seedling emergence studies (Figure 2). The resulting (reduced) survival and growth rates were then proportionally reduced by applying the spray drift exposure level to the dose responses derived from vegetative vigor studies (Figure 2) to obtain the survival and growth rates applied to the simulated *B. decurrens* plants in the model.

### Dose responses of 3 herbicides simulated in the model

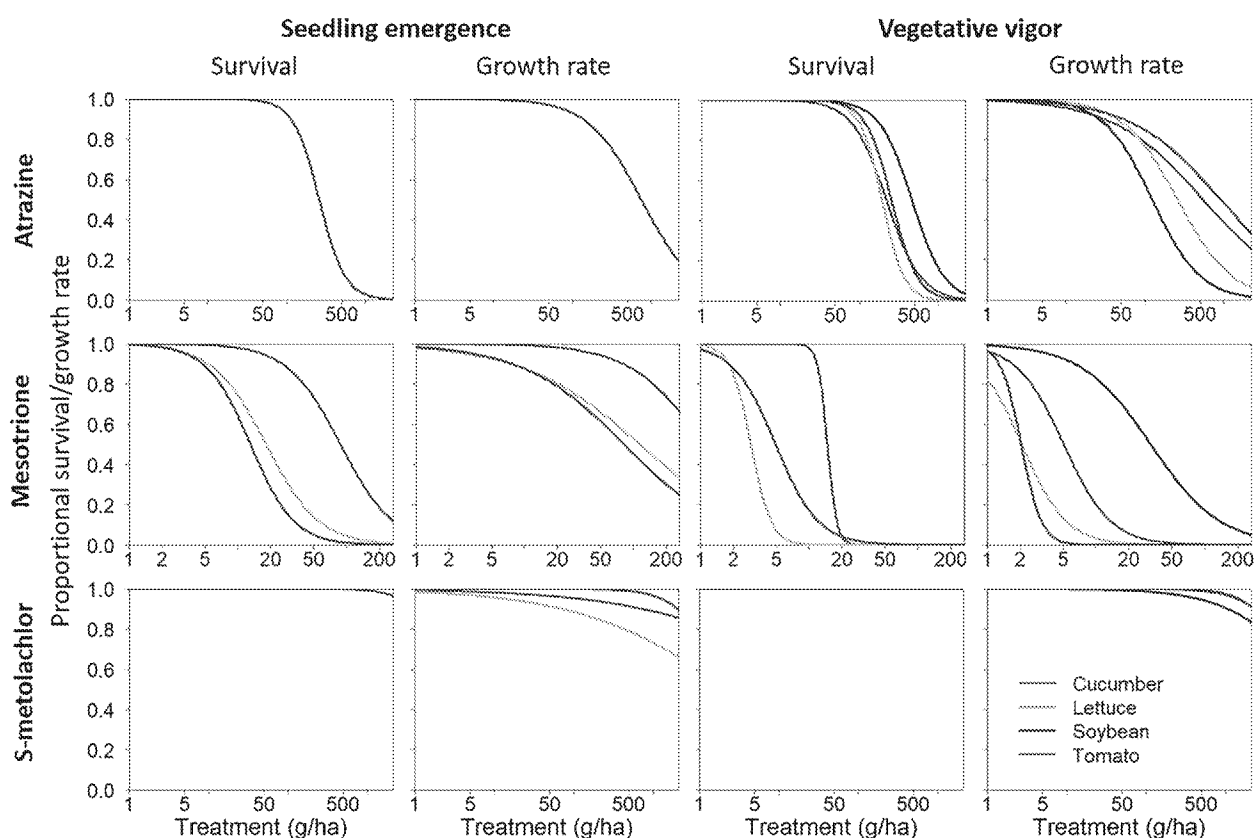
Data from standard seedling emergence and vegetative vigor studies (Organisation for Economic Co-operation and Development 2006a, 2006b; US Environmental Protection Agency 2012a, 2012b) were available for atrazine, mesotrione, and S-metolachlor whereby the active substances were tested, not formulations. Four dicotyledonous plant species were used in all 6 studies: cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*). In the model analysis, we applied the dose responses of each of these 4 species as toxicity scenarios for

*B. decurrens*. We fit log-logistic functions to the data from the studies using nonlinear least squares regression (“nls” in R; R Core Team 2017). In instances where no effect was observed for an endpoint in the studies, it was assumed that the plants do not experience effects in this endpoint for exposure levels simulated in the model. For instance, no reduction in survival rate was observed for cucumber, lettuce, and soybean in the seedling emergence studies of atrazine. In the corresponding toxicity scenarios, the simulated *B. decurrens* plants did not experience a reduction in survival during the seedling stage (first 2 wk of growth) from exposure to atrazine via runoff (which is assumed to affect seedling emergence). None of the 4 test species experienced a significant reduction in seedling emergence from any of the 3 herbicides at the tested concentrations, and accordingly, no effects on seedling establishment were simulated in the experiments shown in the present study.

Growth rates were assessed in the atrazine seedling emergence and vegetative vigor studies (Brain and Hoberg 2016) but not in the studies with mesotrione and S-metolachlor because this endpoint was only reported in the former study. To estimate effects on growth rate, we used the data on shoot dry weight from the studies. In the atrazine studies, an initial shoot dry weight of 0.0001 g was assumed in the seedling emergence studies, reflecting the smallest weight increment as measured in the present study (and the fact that no aboveground shoots were present at the time of exposure). We used this assumption about the initial shoot dry weight in the seedling emergence studies to estimate growth rates from the mesotrione and S-metolachlor studies. In the vegetative vigor studies, the control plant sizes differed between studies (attributable to slightly different ages of the plants at the beginning of the trials). Accordingly, the initial shoot dry weights at the time of exposure, as assessed in the atrazine vegetative vigor study, could not be used in the other 2 vegetative vigor studies. Instead, the daily growth rate of each test species was calculated from the atrazine control data. From the daily growth rate and the average shoot dry weight of the controls at the end of the mesotrione and S-metolachlor studies, we estimated the initial plant weights at the time of exposure. For each study, this estimate of initial plant weight was used to calculate the growth rate from the shoot dry weight data. For details of the estimates of initial plant weights and the estimates of the dose responses, see Supplemental Data (section 2). In Figure 2, we present the dose responses applied as toxicity scenarios in the simulations. The x-axes are scaled to the field application rate of each herbicide according to its label. The full characterization of the dose–response functions is provided in the Supplemental Data (section 2).

### Simulated exposure patterns

For the present study, we assumed a hypothetical habitat at zero distance from a spray application, as may potentially occur next to a treated field, simulating a worst-case scenario with respect to exposure level. The application is assumed to recur once a year during a time window that reflects the time of corn planting, that is, a preemergent herbicide spray application. The herbicide is assumed to be transported into the habitat via spray



**FIGURE 2:** Dose–response curves for atrazine (top row), mesotrione (middle row), and S-metolachlor (bottom row). Dose responses were derived from standard toxicity studies by fitting log-logistic functions to the data from cucumber, lettuce, soybean, and tomato. If no effects were detected in the studies, no effects were assumed in the corresponding endpoint in the model from the herbicide. The maximum extent of the x-axes reflects the field application rate of each herbicide according to its label (2242 g/ha for atrazine, 269 g/ha for mesotrione, 2141 g/ha for S-metolachlor). Survival and growth rates are shown relative to controls.

drift at the time of application. A runoff event is assumed to occur after the spray application. For the simulations detailed in the present study, the runoff event is assumed to occur within a week after the application. Longer intervals between exposure from spray drift and runoff were also tested but did not affect the model outcomes. In alternative experiments, only one of the exposures was simulated to occur, that is, either spray drift or runoff (see *Simulation experiments*, for details).

Three separate numerical exposure models were used to simulate 1) spray drift deposition, 2) chemical transport in runoff from a hypothetical treated agricultural field, and 3) chemical transport across the hypothetical habitat. Chemical exposure in the hypothetical habitat area was calculated by adding spray drift and chemical flux from runoff from the adjacent agricultural field in a spatially distributed manner. For the application rate outside of the habitat, field application rates according to label were used for atrazine, mesotrione, and S-metolachlor (2242, 269, and 2141 g/ha, respectively). Estimates of chemical mass from spray drift deposition were distributed across the hypothetical  $20 \times 20 \text{ m}^2$  habitat using empirical drift deposition curve data (Wolf and Caldwell 2001). From the Wolf and Caldwell (2001) study, a subset of empirical drift deposition data from 5 low-drift nozzles was selected and used to represent spray drift deposition curves. These nozzles were consistent with

end-use product application of a preemergent herbicide where a coarse or very coarse spray droplet composition would optimize maximum coverage of bare soil. Deposition curve data from the following nozzles were included: XR8003, AIXR110-025, DR110-05, TT11005, and A11104. Selected deposition curve data from these nozzles corresponded to wind speed conditions as close to 10 mph as possible (ranged between 7.46 and 10.63 mph) within the limits of the data collected by Wolf and Caldwell (2001). Boom height for these deposition curves was between 60 and 90 cm above ground surface.

The empirical spray drift deposition data from the 5 nozzles were then averaged across 2-m intervals (up to 20 m corresponding to the total length of simulated habitat), creating 2-m calculation intervals/block. Each 2-m calculation interval/block across the hypothetical habitat was assigned a distance-specific, average chemical mass from spray drift deposition corresponding to the associated distance away from the hypothetical upland field edge (where the spray drift deposition fraction would be 1.0). This approach was similar to that used by Schmolke et al. (2017).

We used the Pesticide Root Zone Model (PRZM, Ver 3.1.2 [Carsel et al. 1985]) to represent the cropland bordering the hypothetical habitat. The PRZM model is a one-dimensional simulation framework that was developed to specifically

represent pesticide transport in runoff, vadose, and phreatic zones and is run at a daily time step. Thirty years of meteorological data were simulated in PRZM using the Peoria, Illinois, National Oceanic and Atmospheric Administration weather station (WBAN no. 14842). Of the 30 yr of simulated weather, a single year was carried forward in the population model to represent a conservative exposure profile, that is, the exposure profile from the year represented by the 90th percentile maximum 21-d rolling average and total annual chemical load. The aerobic soil metabolism chemical degradation pathway was explicitly simulated, based on first-order kinetics. For the purposes of the present study, chemical degradation was relevant to simulate, given the relatively long duration of simulated habitat response and differences in chemical degradation rates. Atrazine, mesotrione, and S-metolachlor were assigned estimated chemical half-life values of 57, 17, and 58.2 d, respectively.

On a daily time step, all runoff chemical mass flux and runoff estimates from PRZM were assumed to instantaneously reach the edge of a  $20 \times 20 \text{ m}^2$  hypothetical field and enter a  $20 \times 20 \text{ m}^2$  hypothetical habitat area, represented by the vegetative filter strip model (VFSSMOD; Muñoz-Carpena et al., 1999). The VFSSMOD is a field-scale, mechanistic, storm-based model that is designed to route incoming hydrographs from an adjacent field (in this case, PRZM-generated) through a vegetative filter strip and consists of a series of modules including overland flow, infiltration, water quality/transport, and sediment transport. The hypothetical  $20 \times 20 \text{ m}^2$  habitat in VFSSMOD was divided into 2-m blocks in which chemical mass transport and transformation were explicitly estimated from spray drift deposition and chemical mass transport across the habitat. The resulting habitat exposure model framework could be described as a pseudo-2-d (1-d finite element, effective 2-d in the lateral flow direction), spatially distributed system (assuming Hortonian flow is predominant over 20 m from the edge of the hypothetical upland field) for which chemical mass was estimated for each 2-m increment across the length of the habitat, up to a 20-m distance (total length of the habitat).

### Data collection and analysis from simulations

For each exposure–effects scenario (see *Simulation experiments*, for details), data from 100 simulation runs were collected. Ten different flood scenarios were combined with 10 different herbicide application timing scenarios for the 100 simulation runs. In a previous analysis of the model, the occurrence and duration of floods interacted with the magnitude of pesticide effects, and relative effect sizes of pesticides were more pronounced in the absence of floods and under variable flood scenarios (Schmolke et al. 2017). In the present study, we apply 10 variable flood scenarios as derived from gage readings from New LaGrange Lock and Dam gage station on the Illinois River between 1985 and 2014 (US Army Corps of Engineers 2016; Schmolke et al. 2017). For each flood scenario, a year between 1985 and 2014 was picked randomly for each of the 15 yr of the simulations, resulting in flood scenarios that were variable

between years. The flood scenarios applied are provided in the Supplemental Data (section 3.1).

The timing of herbicide application was derived from the timing of corn planting in Illinois. The week of herbicide application was chosen stochastically from the period during which corn planting occurred in Illinois between the years 2005 and 2014. Weeks with higher occurrence of corn planting were more likely to be chosen than weeks with low occurrence of corn planting (Schmolke et al. 2017). Scenarios applied in the simulations for the present study are listed in the Supplemental Data (section 3.2). Each of the 10 flood scenarios was combined with each of the 10 scenarios of herbicide application timing, resulting in 100 simulations for each experimental setting (see *Simulation experiments*).

In every simulation, the plant populations were allowed to stabilize before the specific treatment was started. This was achieved by a 5-yr “establishment phase” during which beneficial conditions for the species were simulated by preceding each year with 10 wk of winter flooding and no herbicide applications. The densities of flowering plants (number of plants in the habitat divided by habitat area) at the end of each simulated year were collected for the following simulated 15 yr. Simulations were repeated with herbicide applications and without applications (control). From the yearly flowering plant densities, the relative densities were computed by dividing the flowering plant density observed in the exposed habitat by the density in the control habitat. The average of that fraction across the 15 yr of the model run was calculated as the final output of each simulation run.

Reporting *p* values from statistical tests comparing model outcomes collected with different model settings does not inform the interpretation of model results: by definition, we change the population (in a statistical sense) we are collecting data from by changing model parameters or inputs, violating the basic null hypothesis of statistical tests that 2 populations are *not* different (White et al. 2014). Statistical significance based on a *p* value can always be achieved with a model by an arbitrarily high number of repetitions. Instead, the magnitude of differences in model outcomes should be the focus of interpretation and whether the magnitude of difference is biologically relevant. For the analysis of the simulation results from the *B. decurrens* model, we define a reduction in average population density by 5% or more attributable to recurring herbicide applications in comparison to control scenarios as a “relevant” effect. An effect size of 5% represents an arbitrary threshold (as also used for *p* values in statistical tests). However, effect sizes that can be detected in standard toxicity tests generally are more than 5% (Moore and Caux 1997; Hanson et al. 2003). By defining 5% as the level of a biologically relevant effect size, we assume that average reductions in population size by less than 5% would not be detectable in an empirical study and would not affect long-term population persistence.

### Simulation experiments

**Exposure to 3 herbicides via spray drift and runoff.** With the model, we simulated exposure to spray drift from the 3

herbicides (atrazine, mesotrione, and S-metolachlor) to *B. decurrens* populations affecting vegetative vigor and runoff affecting seedling emergence. We assessed the effects on the simulated populations exposed to either spray drift or runoff or a combination of both. When exposed to a combination, runoff was assumed to occur temporally within 1 wk after the spray application. Effects were simulated according to the 4 toxicity scenarios derived from the 4 test plant species. With the simulation of different exposure routes, the relative effects on the modeled plant populations were assessed.

**Introduction of spray application setback distances.** Potential exposures of nontarget plants to herbicides would be highest directly adjacent to a spray application for both exposures via spray drift and runoff. Accordingly, buffers (or spray setback distances) are applied between the spray and the simulated occurrence of the *B. decurrens* populations to test mitigating the potential effects to the plants (Marrs and Frost 1997; Brain et al. 2017). With the model, we tested the effectiveness of different buffer sizes (setback distances) to reduce simulated effects to *B. decurrens* populations growing in a habitat adjacent to a spray application targeting  $\leq 5\%$  difference between control and application scenarios. We applied exposure profiles to the simulated habitat that correspond to spray drift and runoff as experienced by plants if spray application was set back from the spray edge by 0, 4, and 8 m. Setback distances were tested for atrazine and mesotrione toxicity scenarios. For all experiments conducted with the model as shown in the present study, it was assumed that the co-occurring vegetation experiences the same level of effects as the *B. decurrens* plants; that is, the vegetation index is not affected by herbicide exposures.

## RESULTS

### Comparison of exposure routes

The effects of modeled herbicide exposures on simulated *B. decurrens* populations were analyzed as average reductions in population densities in proportion to controls (simulations without herbicide applications). Simulations were conducted with different timings and durations of flooding across the 15 simulated yr, which resulted in considerable variation in absolute plant numbers between years. Flood scenarios influenced the effect size seen at the population level, but the timing of the herbicide application did not have an influence on relative effect size (see Supplemental Data, sections 4.1 and 4.2).

Across flood scenarios, no relevant effect sizes of exposure to runoff were observed in the model for the 3 herbicides tested (effect size  $< 5\%$ ; Figure 3). Effect sizes attributable to exposure to spray drift varied considerably between the 3 herbicides. For atrazine, reduction in population density ranged around 10% in comparison to control levels (Figure 3A). Effect sizes were similar between the 4 toxicity scenarios (dose responses from cucumber, lettuce, soybean, or tomato applied to simulated *B. decurrens* plants) even though organism-level dose responses differed for atrazine.

Effects of simulated spray drift exposure to mesotrione were most pronounced with population-level inhibition of an average

of 30 and 28% for lettuce and tomato dose responses applied to *B. decurrens* plants, respectively. When mesotrione dose responses of cucumber were applied, populations were on average 11% smaller than control populations. No relevant effect size of mesotrione spray drift exposure was observed in the simulations using soybean dose responses (effect size  $< 5\%$ ; Figure 3B). No relevant effect sizes on the populations from exposure to S-metolachlor were observed irrespective of the exposure route (Figure 3C).

When both exposure pathways were simulated to occur in combination, effect sizes were not distinguishable from exposures to spray drift only. Results from the combined exposures are presented in Figure 3 for the 3 herbicides. Different time intervals between exposure to spray drift and runoff were tested with the model, that is, runoff occurring 8 to 14 or 15 to 21 d after spray drift exposure. The time interval between modeled exposure to spray drift and runoff did not change the population-level effects (data not shown).

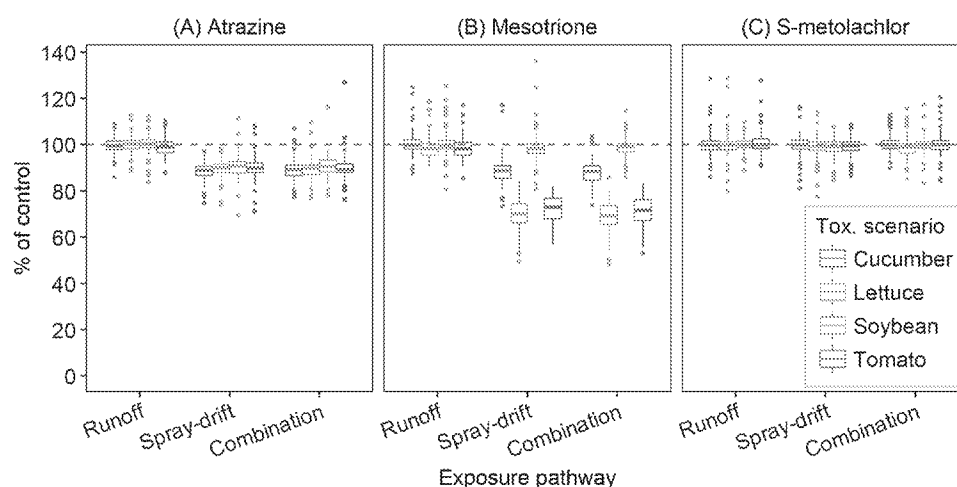
In standard toxicity studies, generally the most sensitive endpoint is reported from all species included in the tests. We calculated the inhibition in each organism-level endpoint for the average exposure level as applied in the model simulations. The most sensitive endpoints (from the 4 test species included) according to the dose responses are summarized in Table 1. The test species with the highest inhibition according to dose responses varied with endpoint and herbicide; that is, none of the test species could be identified as most sensitive to exposure to any of the 3 herbicides across endpoints (Table 1). When comparing organism-level with population-level inhibition, no simple relationship between the endpoints was apparent; that is, organism-level endpoints were not good predictors of population-level outcomes (Table 1). Population-level model outcomes did approach organism-level endpoints for low effect levels as seen for S-metolachlor (Table 1). The complete data set for all 4 toxicity scenarios is presented in the Supplemental Data (section 4.4).

### Simulated spray setback distances

Exposure profiles for spray drift and runoff exposures across the habitat were developed for hypothetical setback distances for herbicide spray applications. Setback distances mean that pesticides are only applied up to a given distance proximal to a species or habitat of conservation interest. For the simulation results presented, it was assumed that setback distances were bare ground rather than ground planted with vegetation (e.g., vegetative buffers). Combined exposures of spray drift and runoff affecting vegetative vigor and seedling emergence, respectively, were simulated for the assessment of effectiveness of setback distances in the instances where any impacts were observed with the model.

In Figure 4, average population densities relative to the control (without herbicide application) are presented for atrazine and mesotrione. Not included in the analysis of setback distances was S-metolachlor because no relevant effect size on populations was observed in the simulations without setback distances (see Figure 3C). The simulation results show that





**FIGURE 3:** Population densities in relation to control (without herbicide application). Simulated *Boltonia decurrens* populations were exposed to runoff affecting seedling emergence, spray drift affecting vegetative vigor, or a combination of both. Exposures profiles applied are based on the assumption that the simulated habitat is directly adjacent to a treated area (no spray setback distance). Three herbicide toxicity profiles were applied: (A) atrazine, (B) mesotrione, (C) S-metolachlor. Each scenario was repeated 100 times with varying flood and herbicide application timings (see section *Data collection and analysis from simulations*). Medians as a percentage of control are shown; boxes delineate the interquartile range, showing the 25th and 75th percentiles. Whiskers extend up to 1.5 times the interquartile range, and outliers are beyond the whiskers.

setback distances can be effective at reducing herbicide exposure and effects on *B. decurrens* populations growing in a habitat next to a treatment. For atrazine, effects in the simulations were mitigated completely with a setback distance of 4 m for all 4 toxicity scenarios tested (effect sizes <5% at 4- and 8-m setback distances; Figure 4A). For mesotrione, no relevant effect sizes were observed on populations with sensitivity to the herbicide according to cucumber if a 4-m setback distance was applied (effect sizes <5%; Figure 4B). If dose responses of lettuce or tomato were applied to the simulated *B. decurrens* plants, an 8-m setback distance resulted in no relevant effect sizes on the populations; that is, the average population reduction was smaller than 5%. If soybean dose responses were applied, no relevant effect sizes of mesotrione on simulated *B. decurrens* populations were observed with or without setback distances (effect sizes <5%; Figure 4B).

## DISCUSSION

With the species-specific population model of the threatened plant *B. decurrens*, we set organism-level effects data from 3

herbicides in a population-level context. Herbicide spray drift and runoff from treated fields affected the simulated plants differently dependent on life stage. Exposure from runoff affected seedling survival and growth, that is, early growth of the plants, whereas exposure from spray drift was assumed to impact survival and growth of established plants in vegetative stages. Simulated populations did not experience relevant effects (that is, reductions in abundances over extended time periods) from exposures to runoff alone irrespective of the herbicide tested. Dose-response relationships from the 3 herbicides showed relatively low plant sensitivity in seedling emergence studies (assessing impacts on germination rate, seedling survival, and growth rates). However, even if simulated runoff exposures to mesotrione were increased 10-fold, the populations experienced either no relevant impacts (for cucumber and soybean toxicity scenarios) or low impacts (7% reduction in population abundance compared to controls for lettuce and tomato toxicity scenarios; see Supplemental Data, section 4.3). Mesotrione exposures were chosen as examples because mesotrione showed the highest sensitivity in survival and growth of the test species out of the 3 herbicides included in the present study.

**TABLE 1:** Comparison of organism- and simulated population-level effects of the 3 herbicides

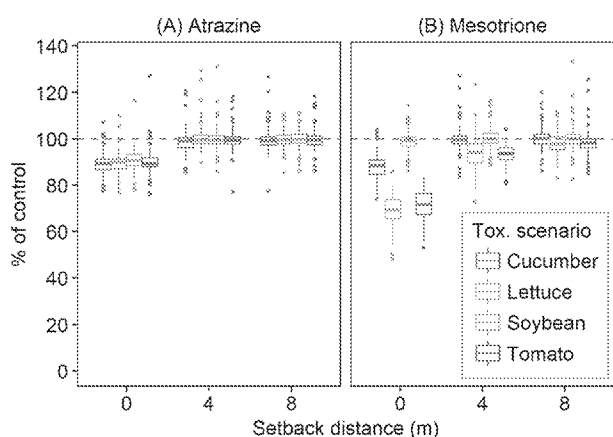
	Organism-level inhibition <sup>a</sup>				Simulated population-level inhibition <sup>b</sup> Population density
	Seedling emergence		Vegetative vigor		
	Survival	Growth rate	Survival	Growth rate	
Atrazine	n.e.	n.e.	7.2% (cucumber)	27.1% (soybean)	10.7% (cucumber)
Mesotrione	10.4% (tomato)	7.2% (lettuce)	70.5% (lettuce)	92.4% (tomato)	31.2% (lettuce)
S-metolachlor	n.e.	7.5% (lettuce)	n.e.	n.e.	n.e.

<sup>a</sup>Organism-level effects were estimated from dose-response functions (fit to data from standard toxicity studies) and using the average exposure applied in the population model. None of the 4 test species showed effects on germination rate in the standard toxicity studies.

<sup>b</sup>For the simulated population-level effects, means of the combined exposures (spray drift and runoff) are stated.

n.e. = no relevant effect size, i.e., effect size <5% for the 4 test species.





**FIGURE 4:** Population densities in relation to control (without herbicide application). Simulated *Boltonia decurrens* populations were exposed to a combination of spray drift and runoff. Exposure profiles corresponding to 0, 4, and 8 m in-field spray setback distances were tested. Two herbicide toxicity profiles were applied: (A) atrazine, (B) mesotrione. Each scenario was repeated 100 times with varying flood and herbicide application timings (see section *Data collection and analysis from simulations*). Medians as a percentage of control are shown; boxes delineate the interquartile range, showing the 25th and 75th percentiles. Whiskers extend up to 1.5 times the interquartile range, and outliers are beyond the whiskers.

The model is based on the assumption as derived from the literature that *B. decurrens* plants are prolific seed producers and that seedlings occur at high densities if conditions are favorable for germination in the spring and early summer (Moss 1997). Intraspecific competition leads to natural thinning of stands as plants grow. Accordingly, reductions in seedling survival or early growth lead to reductions in competition and ultimately in compensation of the effects at the population level. The picture is different when it comes to impacts on survival and growth in later stages. As the intensity of intraspecific competition declines with plant size, reduced survival in vegetative stages ultimately can lead to reduced flowering plant numbers. Impacts from herbicides on plant growth and survival interact with intraspecific competition in the model. Intraspecific competition is explicitly represented in the model as shading between neighboring plants, resulting in density dependence as an emergent property of the model. Reductions in growth are reflected in smaller plants at maturity. In addition to seeds, mature *B. decurrens* plants produce vegetative rosettes that overwinter and reach maturity in the following year (Schwegman and Nyboer 1985). The number of vegetative rosettes produced is dependent on the size of the flowering plant, a relationship observed in the field and included in the model (Baker 1997; Redmond 1993; Tofari 2000). As a result, plant numbers are affected by the number and size of plants present in the habitat in the previous year.

These outcomes are not easily predictable without a model but can be of considerable importance to pesticide risk assessments. For *B. decurrens*, exposures to plants in vegetative stages should be the focus of risk assessments because populations are considerably more sensitive to impacts on survival and growth of vegetative stages rather than germination rate, early seedling survival, and growth. Exposures from spray drift affecting vegetative survival and growth are more likely to affect populations of the plant.

The combination of effects on survival and growth were simulated in the model. Single organism-level endpoints from the toxicity profiles applied in the model were not predictive of the population-level effects. For instance, in the example for mesotrione, lettuce was the most sensitive species in the vegetative vigor study if the survival rate is considered (see Table 1). However, the growth rate of tomato plants showed the greatest inhibition in the same study. The simulation results suggest that *B. decurrens* populations would be most vulnerable to exposures to mesotrione if they had dose responses similar to either lettuce or tomato. There is no evidence that crop plants (as used in the standard toxicity studies) are more or less sensitive to herbicides than nondomesticated plant species (McKelvey et al. 2002; Boutin et al. 2012; Egan et al. 2014b). Accordingly, the range of sensitivities to an herbicide displayed by the test species can be used to explore the potential population-level effects of a nontarget species for which no toxicity data are available.

From the scenarios tested in the model, we only observed population-level effects of exposures to spray drift of atrazine and mesotrione if *B. decurrens* populations were simulated to grow in a small habitat located at the edge of a treated area. A common mitigation strategy to reduce exposures is the introduction of setback distances of spray application (Brain et al. 2017). With the model, we assessed the effectiveness of such setback distances to mitigate effects as observed in the simulations. For the setback distances, bare ground was assumed as would be the case for herbicide applications during planting of a crop such as corn (Brain et al. 2017). The model results suggest that setback distances can be effective at mitigating the potential effect of herbicides on plant populations growing adjacent to a treatment.

Brain et al. (2017) found in a field study that no significant effects resulted on plant metrics from exposures to mesotrione spray drift at a distance of 30 ft (~9 m) from the treated field edge. Potted lettuce and tomato plants were placed at different downwind distances from the edge of a treated field and subsequently assessed according to standard vegetative vigor study protocols. In a microcosm approach, Marrs and Frost (1997) exposed experimental species communities to spray drift of 3 different herbicides in 3 recurring yr. At a distance of 8 m from the spray application, no effects on plant size or health, seed production and viability, or community composition were observed. Accordingly, the present study supports the findings in previous publications that practicable spray setback distances can be effective at mitigating potential effects on listed and other nontarget plant populations that may grow around treated agricultural fields.

## CONCLUSIONS

The population model of *B. decurrens* systematically combines information on the ecology and life history of the species and exposure and effects data for herbicides. The effects of different herbicides can be assessed at the population level, and realistic scenarios can be tested. Accordingly, the model provides a tool that can inform risk assessments of various

herbicides. We could identify spray drift as the more important exposure pathway for the species and herbicides evaluated. The simulation results suggest that populations of *B. decurrens* growing next to fields treated with herbicides could experience impacts from exposures via spray drift. However, potential effects vary with herbicide potency, and introducing spray application setback distances was identified as an effective strategy to mitigate potential effects on populations.

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4093.

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**Data availability**—The model and input data are available in the Supplemental Data. Model outputs and scripts as used for the present study are available on request from A. Schmolke (schmolkea@waterborne-env.com).

## REFERENCES

- Baker MA. 1997. Effects of intra and interspecific rosette competition on *Boltonia decurrens*. MS thesis. Southern Illinois University at Edwardsville, Edwardsville, IL, USA.
- Baskin CC, Baskin JM. 2002. Achene germination ecology of the federally threatened floodplain endemic *Boltonia decurrens* (Asteraceae). *American Midland Naturalist* 147:16–24.
- Boutin C, Aya KL, Carpenter D, Thomas PJ, Rowland O. 2012. Phytotoxicity testing for herbicide regulation: Shortcomings in relation to biodiversity and ecosystem services in agrarian systems. *Sci Total Environ* 415:79–92.
- Boutin C, Strandberg B, Carpenter D, Mathiassen SK, Thomas PJ. 2014. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? *Environ Pollut* 185: 295–306.
- Brain RA, Hoberg J. 2016. Recovery of terrestrial plants in vegetative vigor and seedling emergence tests from exposure to atrazine. *Environ Toxicol Chem* 35:1284–1296.
- Brain RA, Perine J, Cooke C, Ellis CB, Harrington P, Lane A, O'Sullivan C, Ledson M. 2017. Evaluating the effects of herbicide drift on nontarget terrestrial plants: A case study with mesotrione. *Environ Toxicol Chem* 36:2465–2475.
- Brain RA, Teed S, Bang J, Thorbek P, Perine J, Peranginangin N, Kim M, Valenti T, Chen W, Breton R, Rodney S, Moore D. 2015. Risk assessment considerations with regard to the potential impacts of pesticides on endangered species. *Integr Environ Assess Manag* 11:102–117.
- Carsel RF, Mulkey LA, Lorber MN, Baskin LB. 1985. The pesticide root zone model (PRZM): A procedure for evaluating pesticide leaching threats to groundwater. *Ecol Modell* 30:49–69.
- de Snoo GR, van der Poll RJ. 1999. Effect of herbicide drift on adjacent boundary vegetation. *Agric Ecosyst Environ* 73:1–6.
- Egan JF, Bohnenblust E, Goslee S, Mortensen D, Tooker J. 2014a. Herbicide drift can affect plant and arthropod communities. *Agric Ecosyst Environ* 185:77–87.
- Egan JF, Graham IM, Mortensen DA. 2014b. A comparison of the herbicide tolerances of rare and common plants in an agricultural landscape. *Environ Toxicol Chem* 33:696–702.
- Forbes VE, Galic N, Schmolke A, Vavra J, Pastorok R, Thorbek P. 2016. Assessing the risks of pesticides to threatened and endangered species using population modeling: A critical review and recommendations for future work. *Environ Toxicol Chem* 35:1904–1913.
- Galic N, Hommen U, Baveco JMH, van den Brink PJ. 2010. Potential application of population models in the European ecological risk assessment of chemicals. II. Review of models and their potential to address environmental protection aims. *Integr Environ Assess Manag* 6:338–360.
- Grimm V, Augusiak J, Focks A, Frank BM, Gabsi F, Johnston ASA, Liu C, Martin BT, Meli M, Radchuk V, Thorbek P, Railsback SF. 2014. Towards better modelling and decision support: Documenting model development, testing, and analysis using TRACE. *Ecol Modell* 280:129–139.
- Hanson ML, Sanderson H, Solomon KR. 2003. Variation, replication, and power analysis of *Myriophyllum* spp. microcosm toxicity data. *Environ Toxicol Chem* 22:1318–1329.
- Harrison WF. 1988. Endangered and threatened wildlife and plants; Determination of threatened status for *Boltonia decurrens* (decurent false aster). *Fed Reg* 53:45858–45861.
- Marrs RH, Frost AJ. 1997. A microcosm approach to the detection of the effects of herbicide spray drift in plant communities. *J Environ Manage* 50:369–388.
- Marrs RH, Frost AJ, Plant RA. 1991. Effects of herbicide spray drift on selected species of nature conservation interest: The effects of plant age and surrounding vegetation structure. *Environ Pollut* 69:223–235.
- McKelvey RA, Wright JP, Honegger JL. 2002. A comparison of crop and non-crop plants as sensitive indicator species for regulatory testing. *Pest Manag Sci* 58:1161–1174.
- Moore DRJ, Caux P-Y. 1997. Estimating low toxic effects. *Environ Toxicol Chem* 16:794–801.
- Moss JK. 1997. Stage-based demography of *Boltonia decurrens*, a threatened floodplain species. MS thesis. Southern Illinois University at Edwardsville, Edwardsville, IL, USA.
- Muñoz-Carpena R, Parsons JE, Gilliam JW. 1999. Modeling hydrology and sediment transport in vegetative filter strips. *J Hydrol* 214:111–129.
- National Research Council. 2013. Assessing risks to endangered and threatened species from pesticides. National Academies, Washington, DC, USA.
- Organisation for Economic Co-operation and Development. 2006a. Test No. 208: Terrestrial plant test: Seedling emergence and seedling growth test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
- Organisation for Economic Co-operation and Development. 2006b. Test No. 227: Terrestrial plant test: Vegetative vigor test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
- R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Redmond A. 1993. Population study of *Boltonia decurrens*, a federally threatened plant species. MS thesis. Southern Illinois University Edwardsville, Edwardsville, IL, USA.
- Schmolke A, Brain R, Thorbek P, Perkins D, Forbes V. 2017. Population modeling for pesticide risk assessment of threatened species—A case study of a terrestrial plant, *Boltonia decurrens*. *Environ Toxicol Chem* 36:480–491.
- Schmolke A, Thorbek P, Chapman P, Grimm V. 2010a. Ecological models and pesticide risk assessment: Current modeling practice. *Environ Toxicol Chem* 29:1006–1012.
- Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010b. Ecological models supporting environmental decision making: A strategy for the future. *Trends Ecol Evol* 25:479–486.
- Schwegman JE, Nyboer RW. 1985. The taxonomic and population status of *Boltonia decurrens* (Torr and Gray) Wood. *Castanea* 50:112–115.
- Smith M, Caswell H, Mettler-Cherry P. 2005. Stochastic flood and precipitation regimes and the population dynamics of a threatened floodplain plant. *Ecol Appl* 15:1036–1052.
- Smith M, Keevin TM. 1998. Achene morphology, production and germination, and potential for water dispersal in *Boltonia decurrens* (decurent false aster), a threatened floodplain species. *Rhodora* 100: 69–81.
- Smith M, Keevin T, Mettler-McClure P, Barkau R. 1998. Effect of the flood of 1993 on *Boltonia decurrens*, a rare floodplain plant. *Regulated Rivers* 14:191–202.

- Smith M, Moss JS. 1998. An experimental investigation, using stomatal conductance and fluorescence, of the flood sensitivity of *Boltonia decurrens* and its competitors. *J Appl Ecol* 35:553–561.
- Sparks RE, Nelson JC, Yin Y. 1998. Naturalization of the flood regime in regulated rivers. *BioScience* 48:706–720.
- Stoecker MA, Smith M, Melton ED. 1995. Survival and aerenchyma development under flooded conditions of *Boltonia decurrens*, a threatened floodplain species and *Conyza canadensis*, a widely distributed competitor. *American Midland Naturalist* 134:117–126.
- Tofari SM. 2000. A demographic investigation into the life history stage and reproductive biology of *Boltonia decurrens*, a threatened floodplain species. MS thesis. Southern Illinois University Edwardsville, Edwardsville, IL, USA.
- US Army Corps of Engineers. 2016. RiverGages.com: Water levels of rivers and lakes. Washington, DC. [cited 2015 September 15]. Available from: [rivergages.mvr.usace.army.mil/WaterControl/stationinfo2.cfm?sid=IL08&fid=NLGI2&dt=S](http://rivergages.mvr.usace.army.mil/WaterControl/stationinfo2.cfm?sid=IL08&fid=NLGI2&dt=S)
- US Environmental Protection Agency. 2012a. Ecological effects test guideline, OCSPP 850.4100: Seedling emergence and seedling growth. EPA 712-C-12. Washington, DC.
- US Environmental Protection Agency. 2012b. Ecological effects test guideline, OCSPP 850.4150: Vegetative vigor. EPA 712-C-011. Washington, DC.
- US Fish and Wildlife Service. 2012. Decurrent false aster (*Boltonia decurrens*) —5-year review: Summary and evaluation. Midwest Region Rock Island Ecological Services Field Office, Moline, IL.
- White JW, Rassweiler A, Samhoury JF, Stier AC, White C. 2014. Ecologists should not use statistical significance tests to interpret simulation model results. *Oikos* 123:385–388.
- Wilensky U. 1999. NetLogo. Evanston, IL, USA. [cited 2017 September 15]. Available from: <http://ccl.northwestern.edu/netlogo/>.
- Wolf TM, Caldwell BC. 2001. Development of a Canadian spray drift model for the determination of buffer zone distances. Expert Committee on Weeds Annual Meeting, Quebec City, QC, Canada, November 26–29 p 60.